

Animals as Sentinels of Human Health Hazards of Environmental Chemicals

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A workshop titled "Using Sentinel Species Data to Address the Potential Human Health Effects of Chemicals in the Environment," sponsored by the U.S. Army Center for Environmental Health Research, the National Center for Environmental Assessment of the EPA, and the Agency for Toxic Substances and Disease Registry, was held to consider the use of sentinel and surrogate animal species data for evaluating the potential human health effects of chemicals in the environment. The workshop took a broad view of the sentinel species concept, and included mammalian and non-mammalian species, companion animals, food animals, fish, amphibians, and other wildlife. Sentinel species data included observations of wild animals in field situations as well as experimental animal data. Workshop participants identified potential applications for sentinel species data derived from monitoring programs or serendipitous observations and explored the potential use of such information in human health hazard and risk assessments and for evaluating causes or mechanisms of effect. Although it is unlikely that sentinel species data will be used as the sole determinative factor in evaluating human health concerns, such data can be useful as for additional weight of evidence in a risk assessment, for providing early warning of situations requiring further study, or for monitoring the course of remedial activities. Attention was given to the factors impeding the application of sentinel species approaches and their acceptance in the scientific and regulatory communities. Workshop participants identified a number of critical research needs and opportunities for interagency collaboration that could help advance the use of sentinel species approaches. *Key words:* environmental chemicals, FETAX, health hazards, public health, risk assessment, sentinel species. *Environ Health Perspect* 107:309–315(1999). [Online 15 March 1999] <http://ehpnet1.niehs.nih.gov/docs/1999/107p309-315vanderschalie/abstract.html>

Animal sentinels, defined by Stahl (1) as "any non-human organism that can react to...an environmental contaminant before the contaminant impacts humans," offer the possibility of expanding our understanding and response to environmental health concerns. The term sentinel species is often used interchangeably with indicator and surrogate species; however, Stahl (1) defines indicator species as organisms that "respond to environmental contaminants...in particular ways, based on scientifically supportable observations" and surrogate species as organisms used or tested "in place of other organisms for various reasons."

The "Using Sentinel Species Data to Address the Potential Human Health Effects of Chemicals in the Environment" workshop, held 23–24 September 1997 in Frederick, Maryland, provided a forum for exploring issues associated with identifying potential human health effects using sentinel species information. Twenty-two individuals from government, academia, and the private sector participated in the workshop, which was cosponsored by the U.S. Army Center for Environmental Health Research, the EPA National Center for Environmental Assessment, and the Agency for Toxic Substances and Disease Registry (ATSDR).

The first half-day of the workshop featured speakers that provided overview information on the workshop scope and objectives and a historical perspective on sentinel species, issues related to extrapolation from nonhuman species to humans, and selected examples of sentinel and surrogate species. During the next day and a half, participants were divided into two breakout sessions that focused on the use of sentinel species information either from experimental models or from field situations using opportunistic, observational, or experimental approaches. The charge given to breakout session participants was to examine both opportunities and barriers related to the application of sentinel species concepts as they pertain to scientific acceptance, risk assessment, and decision making.

Background and Overview

The use of nonhuman organisms as early warning systems for human health risk is not new. Table 1 provides sentinel species examples beginning in the late 19th century. The miner's canary that was used to warn of potentially lethal carbon monoxide concentrations in coal mines (2,3) is perhaps the most widely known application of

a sentinel animal system for monitoring, whereas the neurobehavioral symptoms displayed by cats that consumed methylmercury-contaminated fish from Minamata Bay in Japan in the 1950s (10) provided a good example of a serendipitous observation that had significance for human health.

More recently, the presence of reproductive abnormalities in fish, birds, and alligators has been attributed to the presence of endocrine-disrupting chemicals in the environment (17,25–29). Despite the lack of direct evidence that chemicals in the environment are adversely affecting people, the potential for these compounds to affect human reproduction and development has been clearly demonstrated by these sentinel populations.

Although advances in the understanding and use of sentinel species have been slow to gain acceptance in human health risk assessment and public health, events over the last few years have provided some impetus to the development of sentinel species models. In 1991, the National Research Council (NRC) Board on Environmental Studies and Toxicology, Commission on Life Sciences published a comprehensive review and evaluation of the usefulness of animal sentinels for assessing environmental health hazards (22).

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Table 1. A timeline of examples of animals as sentinels of environmental toxicants and noteworthy events

Sentinel incidents				
Species (references) ^a	Toxicant	Country	Date	Related events
Canaries (2,3)	Carbon monoxide	England	1870s	
Cattle (4,5)	Smog	England		
Cattle	Fluoride	England	1910s	
Horses	Lead	United States		
Cattle (6–9)	TCE	Scotland		
Cats (10)	Mercury	Japan	1950s	
Birds	DDT	United States		
Cattle	Smog	England		
Chickens (11)	PCBs	Japan	1960s	<i>Silent Spring</i> (12) published (1962)
Sheep (13)	OP agents	United States		
Horses and other animals (14,15)	Dioxin	United States	1970s	NRC Symposium on Pathobiology of Environmental Pollutants: Animal Models and Wildlife Monitors (16) (1979)
Dairy cattle (17,18)	PBBs	United States		
			1980s	Task Force on Environmental Cancer and Heart and Lung Disease established (1981) NAS risk assessment paradigm (19) (1983) NCI report, <i>Use of Small Fish Species in Carcinogenicity Testing</i> (20) (1984)
Sheep	Zinc	Peru		
Alligators (21)	DDT, dicofol	United States	1990s	NRC report, <i>Animals as Sentinels of Environmental Health Hazards</i> (22) (1991) Public Law 103-43 (23) enacted (1993) NIEHS establishes ad hoc ICCVAM (1993) ICCVAM report, <i>Validation and Regulatory Acceptance of Toxicological Methods</i> (24) (1997)
Fish	<i>Pfiesteria</i> toxins	United States		

Abbreviations: TCE, tetrachloroethylene; PCBs, polychlorinated biphenyls; OP, organophosphate; NRC, National Research Council; PBBs, polybrominated biphenyls; NAS, National Academy of Sciences; NCI, National Cancer Institute; NIEHS, National Institute of Environmental Health Sciences; ICCVAM, Interagency Coordinating Committee on the Validation of Alternative Methods.

^aAll references as cited by the NRC (22), except for alligator (21).

This document provided much useful background information for the present workshop. Since 1991, several events, although not specifically directed toward sentinel species issues, have provided impetus in this area.

First, the enactment of the National Institutes of Health Revitalization Act of 1993 (23) directed the National Institute of Environmental Health Sciences (NIEHS) to establish criteria and recommend processes for using scientifically validated alternative toxicity test methods in regulatory decision making.

Second, in 1994 an ad hoc Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) was established by the NIEHS. ICCVAM developed a report that provides guidance for validating and evaluating new toxicity test methods (24).

Third, increasing interest in ecological risk assessment applications has prompted recognition of commonalities with human health risk assessment and discussions of how ecological and human health risk assessments might be integrated (30).

One result of these events has been to encourage consideration of new methods to assess environmental toxicity.

At the workshop, participants adopted a broad view of the use of sentinel species data for human health evaluations that included both experimental and observational perspectives. Sentinel species data could include observations of wild animals in field situations as well as data from experimental studies using surrogate animals. Sentinel animal models could involve mammalian or nonmammalian species, domestic animals, or wildlife. Outcomes of interest included mortality and morbidity, developmental defects and reproductive effects, carcinogenicity, neurotoxicity, immunotoxicity, behavioral changes, and others. Sentinel animal populations could be exposed 1) to a single chemical or a complex mixture, or 2) to different media (e.g., air, water, soil, or sediment) in various locations (e.g., workplace, home, ambient environment, laboratory).

There are several potential advantages associated with using sentinel species as indicators of human health hazards.

Sentinel animals may provide early warning of potential risks before disease develops in human populations. For example, disease latency periods are typically shorter in animals than in humans, or animals may be exposed to higher concentrations that produce overt toxicity. For some toxicants, the biomarkers of exposure and toxic effects are similar in humans and the sentinel animals (e.g., acetylcholinesterase inhibition caused by organophosphorus insecticides). In addition, exposure conditions may be comparable under some circumstances, such as for people and their companion animals.

Given these advantages, several applications for sentinel species information were identified (see "Sentinel Species Applications"). However, impediments to broader acceptance of the use of sentinel species models in both the scientific and regulatory communities were recognized (see "Barriers"). Therefore, the immediate challenge is how to maximize appropriate use of sentinel species information. Additional research and activities that could help expand the use of sentinel species models were recommended.

Sentinel Species Applications

Potential applications identified for sentinel species included monitoring environmental media, identifying new exposures of potential concern as a result of observing changes in wild animal populations, and supporting risk assessment at several points in the process. Although it is unlikely that sentinel species data will be used as the sole determinative factor in assessing human health risks, the data could be useful for a weight-of-evidence approach in risk assessment decisions, for providing early warning of situations requiring further study, or for suggesting potential causes and effects. As discussed in "Barriers," a key consideration for any application is understanding the mechanistic similarities and differences between toxicologic effects in the sentinel species and humans.

Monitoring Applications

Workshop participants considered sentinel species monitoring applications to include observations of resident animal species in the environment as well as deliberate placement of appropriate species for exposure and subsequent observation. Some of the exposure- or effects-related sentinel species data have been collected and utilized in assessments for years, whereas other approaches represent possible future uses. An important activity associated with the successful use of sentinel species monitoring data would be to ensure that agencies, researchers, and others involved in collecting

the data communicate their findings to the appropriate groups, such as agencies and public health officials responsible for decisions about exposure and human health. Examples of uses for sentinel species monitoring information discussed at the workshop include regulatory compliance, complex mixture characterization, evaluation of treatment efficacy or restoration success, identification of deleterious ecosystem changes, and public health decision making.

Regulatory compliance. Chemical residue data from food animals have long been used to assess potential exposures to humans and have provided the basis for regulatory actions when safe levels have been exceeded.

Complex mixture characterization. If toxicity is found in a complex mixture using a test system and end point that may have relevance to human health, this may trigger additional studies such as the toxicity identification and evaluation (TIE) process (31–33). The TIE approach is used to determine the toxic fractions or components contributing to the toxic response of a complex mixture.

Evaluation of treatment efficacy or restoration success. After waste effluents have been treated or hazardous waste sites remediated, there remains a question as to the efficacy of the treatment process. When the problem is focused on one or a few chemicals of concern, the question can be answered with chemical analysis. When, as is frequently the case, a complex chemical mixture is involved, it may be useful to apply biomonitoring approaches using sentinel species. One example is an automated fish biomonitoring system developed by the U.S. Army Center for Environmental Health Research (Fort Detrick, MD). The system is being used to evaluate the ground-water treatment effluent of complex chemical mixtures at an Army Superfund site and to prevent potentially toxic materials from reaching surface waters. The use of continuous biomonitoring was recognized and embraced by state and federal regulators as a means to monitor remediation efforts at the site. The system identifies developing toxic conditions in water by continuously monitoring the ventilation and movement patterns of the bluegill (*Lepomis macrochirus*). Physiological stress to the bluegills, characterized by changes in ventilation and movement patterns, is used as an early warning to identify acute toxicity of the treated ground-water discharge. Continuous monitoring and recording of ventilatory rates, ventilation depth, cough rate, and whole-body movement of eight fish exposed to the effluent is used to identify potentially toxic effluent. When six of the exposed fish are responding with significant departures from

baseline conditions and control fish, an effluent sample is automatically collected for chemical analysis, a remote monitor in the treatment facility control area identifies the problem, and, if necessary, the discharge is diverted to storage tanks until the problem is solved. Use of the biomonitoring system has significantly reduced chemical sampling frequency at the site and has enabled the remediation technology to be implemented ahead of schedule (34).

Even before treatment or remediation operations have been initiated, sentinel species approaches can be helpful in identifying sites of toxicity requiring increased attention, as illustrated by the use of amphibian toxicity tests at a Norman, Oklahoma, landfill by the U.S. Geological Survey. The landfill is a mixed waste site contaminated with high concentrations of ammonia, divalent metals, and organic materials such as benzene, toluene, lindane, and many other organics. The city of Norman sewage outfall also discharges onsite. Several environmental stressors were investigated that might affect amphibian populations, among them climate (35), ultraviolet B radiation (36), and pollution (35). A survey of endemic amphibian populations indicated that smaller bullfrogs lived downstream and larger bullfrogs upstream of the sewage outfall. Amphibians often did not breed onsite and few malformed animals were observed when they did breed in the area (<1%). Native amphibians indicated possible impacted areas. *In situ* toxicity experiments showed mortality and some malformation of endemic toad (*Bufo americanus*) embryos at some sites. Lab experiments with the frog embryo teratogenesis assay—*Xenopus* (FETAX) indicated extensive contamination of ground and surface waters, which correlated well with the *in situ* experiments. The *in situ* bioassays with native amphibians together with the biomonitoring and FETAX data indicated possible impacted areas. Ultraviolet B or the presence of an Aroclor 1254-induced metabolic activation system (for human relevance) failed to make water samples more toxic and a toxicity identification evaluation indicated 50% of the observed toxicity was due to ammonia; the remaining toxicity may be caused by metal contamination. Field and laboratory experiments together indicated the source and type of contamination as well as built a weight-of-evidence argument that defined the nature and extent of the problem. In this study, endemic species served as sentinels whereas lab experiments (FETAX) identified the contaminants.

Identify deleterious ecosystem changes. Reconnaissance and surveillance activities may identify deleterious changes in an ecological system that require corrective

actions and/or indicate potential human health concerns. There are a number of long-term monitoring programs in place to evaluate potential ecological and human health exposures by tracking the status of and trends in chemical residues in biota [e.g., the Mussel Watch program (37)]. Evaluation of ecosystem effects data, such as changes in species composition or trends in population levels of key species, can provide evidence of recovery or increased risk. Ecological changes at the population, community, or ecosystem level do not necessarily imply that human health is at risk, but may indicate that further investigations should be undertaken.

An example of this is the ATSDR Great Lakes Project (38). The Great Lakes constitute the largest body of fresh water on the earth and the surrounding area is home to roughly 36 million people. In the Great Lakes, impaired reproduction has been reported in avian and fish populations (38). Eggshell thinning caused by organochlorine compounds has been well documented and has resulted in egg loss among predatory birds. There is a sense that this trend has been reversed because of the success of recently employed pollution prevention strategies. However, populations of reproducing eagles recently observed along the shores of the Great Lakes are attributable to recruitment of birds from inland populations rather than the proliferation of local populations. These immigrant bird populations exhibit diminished reproductive success after feeding on Great Lakes fish for 2 or more years. Congenital malformations have also been observed in Great Lakes birds, including crossed mandibles of different lengths, supernumerary digits, and club feet. In addition to developmental and reproductive abnormalities, immune effects have been observed in wildlife populations. In a survey of herring gulls and Caspian terns, prenatal exposure to organochlorine pollutants was associated with suppression of T-cell-mediated immunity (38). The greatest effect on immune suppression was associated with polychlorinated biphenyls (PCBs). Other toxic effects include enlarged thyroids and tumors in fish.

Recent epidemiologic studies funded by the ATSDR, along with controlled laboratory studies, complement and continue to build upon these wildlife data gathered over the last two decades (38). For example, the findings of PCB levels in human populations, together with findings of developmental deficits and neurologic problems in children whose mothers ate PCB-contaminated fish, are compelling. The findings of wildlife biologists, toxicologists, and epidemiologists clearly indicate that

populations continue to be exposed to persistent toxic substances and that adverse health consequences are associated with these exposures.

Public health decision making. Regulatory compliance data and data collected to evaluate treatments and restoration activities are used to protect the public, e.g., public drinking water supplies. The sampling scheme typically involves predetermined sampling methods and times and emphasizes problem areas. Surveillance data collected for the purpose of monitoring ecosystems is likely to be collected using a sampling scheme, not necessarily designed to serve as a link between exposure and adverse outcomes in specific populations. By contrast, public health decision making requires more detailed data to link specific well-characterized exposures with a well-defined population. Long before any formal risk assessments can be conducted, priorities for human health research must be established. Data collected through regulatory, evaluation, and surveillance mechanisms could be especially useful in identifying important potential public health issues. For example, the suspected adverse effects in wildlife due to endocrine disruptors have impacted public health research. Another recent example is the fish kills that have occurred on the eastern seaboard of the United States and are the result of algal toxins (10). Why dinoflagellates that have likely always been present in eastern U.S. estuarine waters suddenly proliferate and threaten huge populations of fish and other organisms is unknown. However, the events, particularly of the summer of 1997, have prompted many activities aimed at protecting public health, including closing specific areas to fishing, limiting recreation in local rivers, and allotment of Congressional funding for studies to evaluate potential human health effects of exposure to these marine toxins.

Serendipitous Observations

The most widely known situations where sentinel species served as indicators of potential public health hazards have resulted from serendipitous observations of animals in the wild. Widespread mortality of animals such as fish or birds or the presence of malformed animals attract the attention of scientists, regulators, and the public. In many cases, the cause(s) of observed effects in animals is not readily apparent. In Japan in the 1950s, domestic cats developed "dancing cat fever," which was eventually traced to high mercury concentrations in fish and shellfish in Minamata Bay. The high concentrations were caused by effluents from a nearby factory using mercuric chloride in the production of vinyl chloride (39). More recently, there has been much discussion regarding the

potential human health implications of malformed frogs observed in several regions of the United States and Canada. These observations prompted state and federal agencies to initiate extensive investigations to identify the cause of these abnormalities and to determine if there is any associated human health risks.

Caution in interpreting such ecoepidemiologic events is essential, especially when there is concern over the potential human health implications of the event. There must be biologic plausibility behind an extrapolation from adverse effects seen in animal populations to potential adverse human health outcomes. A fish kill due to small concentrations of residual chlorine in the water has little meaning for effects on humans. In many situations, wild animals may be more highly exposed than humans, or there may not be an exposure pathway leading to humans. Fox (40) offers a number of useful suggestions for evaluating causality using ecoepidemiologic data.

Risk Assessment

Environmental managers who must evaluate situations that may impact human health may not know how to incorporate nontraditional information such as sentinel species data into their decision-making process. This section briefly describes the risk assessment process and suggests ways in which sentinel species data may provide useful input to the process. Although sentinel species data may have relevance to both ecological risk assessment (41–43) and human health assessment, the emphasis here is on human health evaluations.

The human health risk assessment paradigm includes four phases: hazard characterization, quantitative dose–response analysis, exposure assessment, and risk characterization [e.g., (44–46)]. In hazard characterization, available experimental animal and human data are evaluated to determine if an agent causes toxicity and, if so, under what conditions. Dose–response analysis evaluates how effects vary with dose level and accounts for uncertainties associated with interspecies differences in response and other factors. Exposure assessment defines the population at risk and considers the type, frequency, duration, and magnitude of exposure. In risk characterization, effects and exposure information are integrated, and major assumptions, inferences, and uncertainties are summarized. Risk managers use the results of the risk assessment, along with considerations of social, economic, and political factors, in making decisions on risk management.

Workshop participants agreed that sentinel species information could provide useful

supporting information at several points in the risk assessment process. In hazard characterization, sentinel species tests can help screen, prioritize, and focus risk assessment efforts and generate hypotheses for further evaluation. If these tests are used to screen chemicals or environmental media for hazard potential, high sensitivity and a low rate of false positive results are desirable, whereas if the emphasis is on an initial demonstration of nonhazard, tests should have high specificity and a low rate of false negatives. When environmental samples include complex chemical mixtures, screening tests can be used in conjunction with chemical fractionation to characterize the contribution of various components of the sample to the overall toxicity.

Although much of the value of data from sentinel species is in defining the potential hazard associated with exposure to environmental chemicals, there are situations in which sentinel animals may be useful in exposure assessment. Exposure assessment can be strengthened using biomarkers common to animals and people. Also, animals can be used to monitor the bioavailability and bioaccumulation of chemical contaminants, e.g., in sediments and foods such as fish. Finally, animals integrate exposures across their entire environment, and appropriate animal sentinels may represent the quality, as well as quantity, of exposure experienced by people.

For risk characterization, a three-pronged weight-of-evidence approach was suggested that relies on sentinel animal data along with more conventional toxicologic and epidemiologic information. In addition, the value of sentinel species data in a weight-of-evidence approach was recognized. For example, there was discussion of the use of sentinel species data along with human epidemiologic data to help assess the human health risks of persistent bioaccumulative chemicals in the Great Lakes (see "Monitoring Applications"). Companion animals (e.g., pets such as dogs) have been useful in a number of instances in providing valuable supplemental information relevant to human diseases. Companion animals often share a common environment with people and may have limited geographic mobility. They are relatively free from many lifestyle and other factors that act as confounders in studies of people and in many cases have similar biological responses to toxic chemicals, but with shorter latency periods. The potential usefulness of companion animals is discussed here [see also Reif et al. (47–49) and Glickman et al. (50)].

Several models of naturally occurring cancers in companion animals have been described, including cancers that are associated with exposure to pesticides. The risk of developing canine malignant lymphoma

in association with exposure to lawn herbicides was assessed in a hospital-based case-control study (51). Cases were 30% more likely to have lived in a home where the owners applied 2,4-dichlorophenoxyacetic acid (2,4-D) or employed a commercial lawn care company to treat their yard. The risk rose to a twofold excess when dog owners used four or more yearly applications of the herbicide and a statistically significant trend was found for the number of applications. Reynolds et al. (52) used biomonitoring techniques to determine the extent to which dogs absorb and excrete 2,4-D in urine after contact with lawn herbicides. Dogs living in and around residences with recent 2,4-D treatments excreted measurable amounts of the herbicide through normal activities and behaviors for several days after exposure, supporting the methods and findings of the Hayes et al. (51) study.

Hayes et al. (53,54) conducted a series of studies based on the Vietnam service of military working dogs and mortality from seminoma. These dogs were potentially exposed to pesticides (picloram, malathion), phenoxyacid herbicides (2,4-D, 2,4,5-trichlorophenoxyacetic acid), zoonotic diseases, and therapeutic agents, particularly tetracycline. When the frequency of autopsy-proved testicular cancer was compared between dogs that had served in Vietnam and those which had remained in the United States, the risk of seminoma was approximately doubled among those dogs that had served in Vietnam. Testicular degeneration, atrophy, and/or oligospermatogenesis was also diagnosed more often in dogs that served in Vietnam [odds ratio (OR) = 1.7]. Identification of an increased risk for seminoma among military working dogs led to a human case-control study to determine whether Vietnam veterans had experienced an increased risk for testicular cancer (55). Service in Vietnam was reported more frequently among cases of testicular cancer than among age-matched controls (OR = 2.5 for Vietnam veterans).

When risk assessment results are used for risk management decisions, scientific information must be communicated to the public, and sentinel species data can be very effective in this regard. For example, the automated fish biomonitoring system described in "Monitoring Applications" has been accepted by the public as a way to ensure that treated groundwater entering a tributary of the Chesapeake Bay does not contain acutely toxic chemicals. However, in some cases responses observed in sentinel species models may have little or nothing to do with potential human health effects; therefore, considerable care is needed in

interpreting such data. For example, aquatic organisms are affected by toxicologic mechanisms (e.g., gill damage by low levels of residual chlorine) or exposure routes (e.g., consuming sediments) that are not relevant to humans. The "Barriers" section describes many of the issues requiring consideration when sentinel species data are applied in the context of human health risks.

Research Tools

Adverse changes observed in animal populations can be useful in generating hypotheses concerning the potential causes of these events, and sentinel species test systems can help evaluate those hypotheses. At the workshop, a number of incidents of malformations in frog populations were described. These malformations have been noted in Minnesota and other states. The nature and pattern of occurrence of malformations have been helpful in generating hypotheses concerning possible causes of the defects, such as parasites, ultraviolet radiation, or xenobiotic chemicals. Laboratory models—such as developmental toxicity tests with native frog species or the FETAX—have been very useful for testing and evaluating hypotheses. Although the human health implications of the observed malformations are unknown, sentinel species observations and testing offer useful approaches for investigating the problem.

Barriers

Optimal use of information and data from sentinel species for regulatory actions, remedial action goals, or public health decisions has been limited by various factors. Barriers that have restricted the use of sentinel species data for evaluating human health implications of environmental contaminants include scientific issues, institutional processes, communication, and economics.

Scientific barriers. A key issue in the evaluation of sentinel species data is understanding of the mechanistic relevance of observed effects to humans. Rather than applying the sentinel concept to a species or organism, it is preferable to consider a specific sentinel end point accompanied by some mechanistic understanding of the observed effect. For example, consider the recent evidence of widespread occurrence of malformed frogs. Limb development is a well-studied phenomenon with highly conserved signaling processes across all vertebrates. Knowing the mechanisms of limb development allows us to assess the plausibility that the environmental effects on limb development may have potential importance with regard to humans or other animals. In addition, knowledge of the relevant mechanisms permits the formation of testable hypotheses that will lead to

elucidating the cause-effect relationship. In contrast, finding meaning in a mass die-off of amphibians (which has also occurred) is more problematic. These observations are far more generic and the mechanisms too diverse to focus on the issue of extrapolation. Therefore, there is little evidence to suggest that a frog die-off is relevant to humans.

It is clear that data from human populations, when available, are preferable to sentinel species information and data from laboratory animals. However, because of inadequate sample size, confounding variables, or other reasons, definitive human data are seldom available to adequately evaluate potential human health risks from environmental contamination. Thus, as noted previously, sentinel species data may contribute significantly to the weight-of-evidence evaluations used for such assessments. Even so, scientists and regulators must have confidence in data obtained from sentinel species, and this requires a diligent assessment of the reliability and relevance of such data. The usefulness of sentinel methods could be further strengthened by expanded information on organism biology and responses, and standardization of test response benchmarks, methods, and reports. The recently developed ICCVAM guidance for the validation of alternative methods (24) should be helpful in facilitating the evaluation of new methods and enhancing the likelihood that validated methods will be accepted and applied (see additional discussion in "Institutional Barriers").

Compilation and evaluation of sentinel species data would be greatly facilitated by the existence of a centralized database. Although certain focused databases exist [e.g., the registry of tumors in lower animals at George Washington University (56)], workshop participants and the NRC both identified the lack of a centralized database for ecological effects as a deficiency. Among the NRC recommendations are the standardization of information systems and electronic media as a means of facilitating the coordination and collection of exposure and disease data from fish, wildlife, companion animals, and livestock (22). This kind of systematically organized information would facilitate data synthesis and analyses that could detect patterns of change through time.

Exposure relevance is critical to comparisons between sentinel animals and humans. Exposure pathways important for sentinels may or may not have any relevance for human populations. If one believes that chemically induced cancer in a bottom-feeding catfish exposed to contaminated sediments is a concern, demonstration of potential human risk would be incomplete

without identification of a plausible exposure route to the human population.

Perhaps the key problem regarding the use of sentinel species data is extrapolation to humans, and there are a number of dimensions to this issue. Qualitatively, there needs to be some assurance that the end point or marker observed in a sentinel species is applicable to humans. Does the occurrence of chemical-related cancer in fish or malformations in frogs have relevance and a plausible link to humans? If this is true, what kind of inferences can be derived concerning dose-response relationships? The answer to both of these questions requires an understanding of the underlying mechanisms of toxicity in both the sentinel animal and in humans. The lack of such mechanistic understanding is a major limitation of many sentinel models.

There are limitations in the interpretation of epidemiologic studies involving either humans or animals. For both human epidemiology and animal ecoepidemiologic studies, adequate statistical power is of prime importance. Enough information about variability and confounding factors for the study population must be known so that an appropriate statistical design can be used. Limited historical data may make it difficult to separate short-term fluctuations from longer term trends occurring in a given sentinel species, population, or community over time. Appropriate control and/or reference sites are critical components of high-quality sentinel species investigations.

Institutional barriers. A number of institutional barriers exist that impede the adoption of well-developed sentinel species approaches for use in environmental decision making. Sentinel species methods, whether laboratory test methods for new chemicals or field ecoepidemiologic studies, must be amenable with existing regulatory frameworks of state and federal agencies. Such methods would need to be relevant to regulations and policy and be able to overcome the considerable inertia in most agencies that resists the adoption of new techniques. For laboratory based test methods, the ICCVAM has provided a standardized process to overcome institutional barriers through the establishment of criteria for the validation and acceptance of new alternative test methods such as sentinel systems (24). The ICCVAM report stresses the importance of early and frequent communication and coordination between regulatory agencies, the developers of new methods, and other stakeholders (e.g., researchers, users, and the public) as new methods are developed, validated, and implemented in regulatory programs.

Other barriers. Other barriers to the use of sentinel species methods involve communication and economic issues. There is a need for improved communications among stakeholders concerning the application, value, and limits of sentinel species methods. This will be necessary to overcome the considerable resistance to the incorporation of nontraditional methods into the environmental decision-making process. It is further recognized that the development and validation of new approaches will require significant resources. The key question is whether the value added by sentinel species approaches will justify additional costs associated with test development and validation as well as the consideration of such ecotoxicological information in human health risk assessments. Initially, it may be easier to achieve incorporation of focused screening tests and support for applied research directed to a limited number of specific issues than to attempt to initiate a broad, long-term sentinel species monitoring and assessment program.

Recommendations

Workshop participants identified research, development, and other activities that would strengthen the usefulness of sentinel species approaches. Recommendations derived from these discussions are as follows:

- Develop biomarkers of exposure and toxic effects that reflect similar biological events in both humans and a sentinel species.
- Conduct joint ecoepidemiologic and human epidemiologic surveys so that data can be simultaneously collected and compared.
- Evaluate the usefulness of sentinel species methods for assessing toxicity of chemical mixtures.
- Elucidate mechanisms of toxicity for environmental chemicals of particular concern.
- Conduct pharmacokinetic studies of chemicals of concern that would facilitate comparisons between sentinel species and humans.
- Expand the toxicity end points assessed by sentinel animal systems (e.g., include neurobehavioral and genetic effects as well as effects on growth and development and the endocrine and immune systems).
- Improve the collection and use of data for a broad range of sentinel species applications.
- Improve communication among government agencies, researchers, and public health officials regarding the availability, interpretation, and application of animal sentinel data and methods.
- Whenever possible, incorporate sentinel animal data into the human risk assessment process.
- Educate regulators on the existence of sentinel animal databases, such as reportable disease-tracking systems for domestic animals and animal tumor registries.
- Educate the public on the information that sentinel animal systems can contribute to the knowledge base on human health and environmental exposures.
- Encourage researchers and developers using sentinel animal systems to integrate and compare data sets from different sentinel animal systems and traditional systems.
- Enhance current sentinel animal systems and generate new methods for assessing toxicologic end points.
- Use tumor registries and reportable disease-tracking systems for domestic animals in examining geographic trends in disease prevalence and incidence.
- Use the integration of survey and monitoring data to examine trends in the types and quantities of contaminants in specific areas.
- Establish a rapid-response team of experts to investigate disease epidemics and morbidity and mortality in wildlife to determine if the cause was related to environmental contaminants.
- Evaluate new methods for assessing toxicity under a specific regulatory process, such as ICCVAM, to ensure that the method generates high quality data useful for human risk assessment.
- Enhance communication among government agencies, researchers, the public health community, and lay citizens to collect, assess, and apply data from sentinel animal systems.

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